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ELECTROPHORETIC VARIABILITY IN ISLAND POPULATIONS OF *DROSOPHILA SIMULANS* AND *DROSOPHILA IMMIGRANS*

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ABSTRACT

Genetic structure and variability was investigated in several Hawaiian populations of D. simulans and D. immigrans. Genetic variability is at intermediate levels when compared to endemic species of Drosophila. Reasons why this variability is higher in island populations of D. simulans than in some continental populations are explored. For D. immigrans, Hawaiian and Korean populations are similar in variability, allelic content and gene frequencies. It is suggested that continual but rare recolonization events in cosmopolitan species can offer an evolutionary advantage over endemic species as it enables a new source of genetic variation.

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INTRODUCTION

Recently, many studies of genetic variability in a broad number of drosophilid species have been reported (Lewontin and Hubby 1966, Johnson 1971, Berger 1971, Prakash et al. 1969, Lakovaara and Saura 1971, Richmond 1972, Ayala et al. 1972). These studies have complemented those dealing with chromosomal variation (for reviews of the latter see Carson 1965, Dobzhansky 1970).

Though the relationships between variable gene loci and variable gene sequences are not yet delineated, it appears that estimates of high chromosomal and genic polymorphism tend to occur together in the same species. First observed by Lewontin and Hubby (1966), this trend seems supported by the findings of O'Brien and MacIntyre (1969) and Berger (1970). The latter two studies have found low or non-existent electrophoretic variation in the chromosomally monomorphic species Drosophila simulans, although Kojima et al. (1970) found evidence to the contrary.

Documented results such as these must have some impact on current evolutionary theory. Estimates of high genetic variability in species such as Drosophila obscura (Lakovaara and Saura 1971) could support arguments suggesting that variable genotypes may aid in preadapting the organism to new or foreign environments. However the apparently low levels of variability in D. simulans, a prototype of the cosmopolitan species, argues for the existence of a general, all purpose genotype (Carson 1965). Such a genotype must, by necessity, be capable of meeting environmental vicissitudes without resorting to calling up genetic reserves inherent within the species gene pool.

In this report, we would like to extend the observations of genic variability in natural populations of D. simulans. Because other observations on this species have concerned continental populations, we thought it would be of interest to compare insular populations having some high degree of isolation. We also include data for the cosmopolitan species, D. immigrans, and compare genic variability in island versus Asian populations. These species are considered to be colonizers in the areas sampled, a point having some bearing on arguments concerning adaptation to new environments.

MATERIALS AND METHODS

Populations studied: The samples of D. simulans and D. immigrans from the island of Hawaii were taken along the Mauna Loa Strip Road, Volcanoes National Park. The

D. simulans samples were collected in October, 1972 at the following elevations: 1212 m elevation (Tree molds area, TM); 1212 m elevation (Kipuka Puaulu, KP); 1273 m elevation (Kipuka Ki, KK); 1591 m elevation (edge of road, designated 1591); and 2121 m elevation (Mauna Loa Crater Trail, designated 2121). One sample of D. simulans was collected in November, 1973 from the Poamoho Trail area of Oahu (about 800 m elevation, designated PT).

The D. immigrans samples from Hawaii were collected in September, 1973 as follows: 1212 m elevation (Sulfur Bank, SB); 1212 m elevation (Kipuka Puaulu, KP); and 1591 m elevation (along edge of road, designated 1591). Drosophila simulans and D. immigrans are sympatric in all of these areas. One sample of D. immigrans was collected at Chunnam National University, Kwangju City, Chunnam Province, Korea by Professor K. W. Kim in October, 1973 and forwarded via mail to Hawaii for chromosomal and electrophoretic assay of genetic variability. The Korean sample suffered over 50% loss of adults during shipping. Collections were normally made using yeasted and fermented fresh banana baits placed in eight quart plastic buckets with an aluminum foil cover to protect against rain. The flies were collected by sweeping over the buckets with a 30 cm insect collecting net and were transported on cornmeal or sugar media (Spieth 1966) to the Department of Genetics, University of Hawaii, for genetic analysis. Although D. melanogaster, a close sibling species of D. simulans, is known to occur at extremely low frequencies in both Kipuka populations, flies were classified via the basis of differences in either male genitalia or in both sexes by differences in allozyme alleles. After verification the samples were frozen at -28°C until such time as electrophoresis could be performed. Live controls on the gels indicated no effect due to storage temperature on the banding patterns.

Gel and electrode buffers: Two buffer systems were used and are described as follows: (A) gel buffer: 74 mM Tris and 8 mM citric acid (monohydrate), pH 8.55; electrode buffer: 687 mM Tris and 157 mM citric acid (monohydrate), pH 8.15, diluted 1:1 (V/V) with distilled water for the cathodal tray and 2:3 (V/V) for the anodal tray. (B) gel buffer: same as above; electrode buffer: 300 mM boric acid and 60 mM NaOH, pH adjusted to 8.55 (modified from Poulik 1957).

Gel preparation: A 12% (W/V) solution of electrostarch (Lot 146) is heated with vigorous swirling to the boiling point in a 1000 ml Erlenmeyer flask. The starch solution is then degassed with an aspirator and poured into a plexiglas mold 19.5 x 17.5 x 1 cm (approximate volume, 425 ml). The gel is cooled for 1½ hours

then covered with Saran Wrap and allowed to cool further for at least 2 more hours.
Sample preparation and electrophoresis: Samples were prepared and placed in the gels similar to the technique of Ayala et al. (1972) with two exceptions: a 1.5 mm cutting guide was used in order to obtain at least 5 slices for staining and Whatman no. 3 filter paper was used to soak up the protein samples for placement in the gels. Electrophoresis was then carried out overnight at either 50 mA per gel (buffer system A) or 35 mA per gel (buffer system B).
Enzyme assays: Protein assays were modified after Ayala et al. (1972) or Selander et al. (1971). The following enzymes were assayed on buffer system A: alcohol dehydrogenase (ADH), glucose-6-phosphate dehydrogenase (G-6PDH), α-glycerophosphate dehydrogenase (α-GPDH), isocitrate dehydrogenase (ICDH), malic enzyme (ME), malate dehydrogenase-1 (MDH-1), and xanthine dehydrogenase (XDH). On buffer system B were assays: phosphatases (ACPH and APH), aldehyde oxidase (ALDOX), esterase-5 (EST), leucine aminopeptidases (LAP), glutamate oxaloacetate transaminases (GOT), octanol dehydrogenase (ODH) and phosphoglucomutase (PGM).

The genetics for the majority of these systems have been previously demonstrated in D. simulans or its close sibling, Drosophila melanogaster (see Courtwright 1966, Fox 1971, O'Brien and MacIntyre 1969, 1968). Allele designations are as in Ayala (1972).

RESULTS

Drosophila simulans variability: The results of the electrophoretic assays for D. simulans are presented in Tables 1-5. Two alleles, one medium and one slow migrating, are found at the ACPH locus (Table 1). A chi-square test for independence between all samples proved insignificant between all samples and each population met Hardy-Weinberg expectations. Kojima et al. (1970) report four alleles for this enzyme. At the PGM locus, (Table 2) the TM population was not in Hardy-Weinberg equilibrium. The finding is due to a lack of 1.00/1.02 heterozygotes and an excess of 1.00/1.00 homozygotes. This did not affect the test for independence between populations in phenotype frequencies, however, and within Hawaii and between islands tests proved insignificant. Although we find three alleles, Kojima et al. (1970) report only two in their study.

At the EST-6 locus (Table 3) we find only two alleles compared to four found by Kojima et al. (1970). The two fast migrating alleles appear to be missing in

Table 1. Gene frequency data for the ACPH locus in *D. simulans*.

Island	Collection	N	Allele Frequency 1.00	.98
Hawaii	KK	114	.728	.272
	KP	46	.728	.272
	TM	118	.754	.246
	2591	69	.732	.268
	2121	26	.673	.327
Oahu	PT	45	.667	.333

Table 2. Gene frequency data for the PGM locus in D. simulans.

Island	Collection	N	Allele Frequencies 1.02	1.00	.98
Hawaii	KK	60	.083	.917	--
	KP	47	.064	.894	.042
	TM*	60	.150	.825	.025
	1591	74	.054	.912	.034
	2121	26	.039	.942	.019
Oahu	PT	48	.062	.938	--

*$\chi^2 = 27.21$, $P < 0.01$

Table 3. Gene frequency data for the EST-6 locus in D. simulans.

Island	Collection	N	Allele Frequency 1.00	.98	% EST-6 homozygote in sample
Hawaii	KK	117	.889	.111	1.7%
	KP*	39	.782	.218	12.8%
	TM	119	.853	.147	3.4%
	1591	74	.851	.149	1.3%
	2121	26	.942	.058	None
Oahu	PT	42	.667	.333	16.7%

* $\chi^2 = 7.64$, $P < 0.01$

Table 4. Gene frequency data for the ALDOX locus in D. simulans.

Island	Collection	N	Allele Frequency 1.00	1.02	1.04
Hawaii	KK	57	.640	.281	.078
	KP	35	.600	.343	.057
	TM	68	.667	.246	.073
	1571	77	.663	.240	.097
Oahu*	PT	27	.883	.130	.037

* $\chi^2 = 40.25$, $P < 0.005$

Table 5. Gene frequencies for rare alleles occurring in the Hawaii and Oahu collections of *D. simulans*.

Collection	N	locus	allele	frequency	No. of heterozygotes
KP	29	ADH-1	1.00	.983	1
			1.03	.017	
2121	26	ADH-1	1.00	.942	1
			.97	.058	
2121	26	ME	1.00	.981	1
			1.01	.019	
PT	49	α-GPDH	1.00	.929	3
			.97	.071	

the Hawaiian samples if we assume that the allele with the highest frequency is the same in continental and island species. The same phenomena exists for the ACPH locus. The observation is dependent, however, on verification of allele homologies in the geographic types but evidence indicating that the homologies are true is found in the observation that the allele frequencies for the most commonly occurring allele are similar at the ACPH, PGM and EST-6 loci. For the EST-6 locus, the KP population is not in Hardy-Weinberg equilibrium. The observation is due to an excess of homozygotes for the slow migrating (.98) allele (12.8%, compared to a range of 0-3.4% for the other Hawaii samples). The excess of this phenotype also leads to a significant test for independence ($\chi^2 = 17.91$, $P < 0.02$) for the pooled Hawaii samples. The Oahu sample also differs from the Hawaii samples with the exception of the KP sample which it closely resembles in frequency of the .98/.98 homozygote ($\chi^2 = 27.42$, $P < 0.01$).

For the ALDOX locus, we observe 3 alleles. The Oahu sample is not in Hardy-Weinberg equilibrium, primarily because of an excess of 1.02/1.04 heterozygotes and 1.02/1.02 homozygotes. The result may be due to a sampling error since the sample is quite small. Again this leads to a significant chi-square test for independence between phenotype frequencies in the Oahu and Hawaii samples ($\chi^2 = 20.70$, $P < 0.005$). The observation is subject to the same criticism; however, the high frequency of the 1.00 allele indicates that other reasons may exist if the observation should be verified.

Low levels of sporadically occurring variability were also observed at the ME, ADH-1 and α-GPDH loci (Table 5). Only that for the α-GPDH locus has any significance since sample sizes are sufficient to indicate that allele .97 occurs on Oahu and not on Hawaii, the locus thus being polymorphic on Oahu. Kojima et al. (1970) find that the α-GPDH and ME loci are monomorphic. They observe four alleles for the ADH locus in the population they sampled, with three of these slow migrating and rare types. We observe three, one of which is a fast migrating type. We think it unsafe to draw allele homologies between the allele types in the island and continental forms since the most commonly occurring allele in the widely-separated geographic populations may not be the same. Gibson (1970) has provided evidence why we might expect to see differences in allele frequencies at this locus. Vigue and Johnson (1973) have demonstrated significant differences in allele frequencies which demonstrate a North-South cline.

Table 6 demonstrates the observed heterozygosity estimates for each locus in each population as well as the locus and population averages (calculated after

Table 6. Observed heterozygosity estimates for island populations of D. simulans. Expected estimates are in parentheses.

Locus	KK	KP	TM	1591	2121	PT	locus average
PGM	.133	.213	.100	.149	.100	.083	.132
	(.152)	(.194)	(.297)	(.164)	(.110)	(.116)	
EST-6	.188	.179	.227	.270	.133	.333	.223
	(.197)	(.340)	(.251)	(.254)	(.109)	(.444)	
ACPH	.333	.283	.373	.362	.357	.400	.352
	(.396)	(.396)	(.371)	(.392)	(.440)	(.444)	
ALDOX	.491	.286	.441	.348	NI	.185	.367
	(.414)	(.519)	(.387)	(.494)	(NI)	(.288)	
ADH-1	m	.034	m	m	.038	m	.012
		(.033)			(.109)		
ME	m	m	m	m	.038	m	.006
					(.037)		
α-GPDH	m	m	m	m	m	.062	.016
						(.132)	
population average[a]	.079	.075	.084	.077	.055	.075	.077

[a] based on all loci analyzed for that population

NI not investigated

m Monomorphic

Lewontin and Hubby 1966). The lack of heterozygotes combined with the excess of homozygotes for the most common allele leads to a depressed heterozygosity estimate for the TM population at the PGM locus. Similar observations exist for the EST-6 locus, KP population and the ALDOX locus, PT population. Slight deviations from expected are observed at other loci. It is obvious from this table that island populations of D. simulans are not lacking in variability. Although the estimates are lower than those made for the Austin, Texas population (Kojima et al. 1970), they are higher than those made for other continental populations (Berger 1971, O'Brien and MacIntyre 1969). We will return to a discussion of this point later.

Drosophila immigrans variability: Gene data for D. immigrans is presented in Table 7. No outstanding differences are found in allele frequencies or phenotype frequencies between the Hawaii samples with the exception of the LAP-2 locus where differences in phenotype frequencies approach significance. The populations all appear to be in Hardy-Weinberg equilibrium.

The Korean population of D. immigrans is remarkably similar to the Hawaii populations, especially in allele frequencies. Although sample sizes are not particularly large, they indicate that the populations are similar in allelic content as well.

Table 8 displays the genetic variability estimates for populations of D. immigrans. The estimates are similar in all populations although some differences in average number of alleles per population are also reflected in the population heterozygosity ($\bar{H}$) estimates.

In Table 9 we see the comparisons between the amounts of enzyme variablity in the endemic versus cosmopolitan species. We cannot say which species of introduced Drosophila has the larger population size, but it appears that their amounts of variability are on a par with endemic species. D. mimica and D. engyochracea, which occur in Kipuka Ki and Kipuka Puaulu only, have levels of variability which are higher than sympatric populations of both introduced species (Steiner 1974).

DISCUSSION

The discovery that D. simulans has higher levels of variability than two published reports for continental populations (Berger 1971, O'Brien and MacIntyre 1969) is difficult to explain. The fact that continental populations of

Table 7. Gene frequency data for island and Korean populations of D. immigrans.

		1591		KP		SB		Korea	
Locus	Allele	Freq.	N	Freq.	N	Freq.	N	Freq.	N
ACPH	1.02	.232	99	.261	111	.282	103	.061	41
	1.00	.768		.739		.718		.939	
APH	1.02	.045	56	.224	96	.062	24	.147	17
	1.01	.098		--		--		--	
	1.00	.857		.769		.938		.924	
	.99	--		.007		--		.029	
ALDOX-1	1.02	.013	37	.019	78	.005	92	--	24
	1.00	.919		.949		.962		.917	
	.98	.068		.013		.033		.083	
	.96	--		.038		--		--	
ALDOX-2	1.02	--	0	.083	79	.038	92	.013	39
	1.00	--		.861		.891		.953	
	.98	--		.076		.071		.034	
EST-1	1.00	--	0	.989	98	.995	99	.897	39
	.99	--		.005		.005		.038	
	.98	--		.005		--		.065	
EST-2	1.00	--	0	.985	34	1.00	30	1.00	40
	.99	--		.015		--		--	
EST-6	1.00	--	0	.991	106	.979	47	1.00	41
	.98	--		.009		.021		--	
LAP-1	1.01	--	0	.017	29	--	4	--	21
	1.00	--		.966		1.00		.952	
	.99	--		.017		--		.048	
LAP-2	1.01	.144	90	.250	52	.036	28	.184	38
	1.00	.756		.654		.714		.789	
	.99	.100		.096		.250		.026	
GOT-1	1.03	.005	91	--	0	--	0	--	0
	1.00	.989		--		--		--	
	.97	.005		--		--		--	
GOT-2	1.03	.038	40	.075	20	.031	32	.061	41
	1.00	.925		.900		.953		.927	
	.97	.012		.025		.016		.012	
ODH	1.00	.966	29	.978	92	.983	87	.979	24
	.92	.034		.022		.017		.021	
PGM	1.02	--	8	.013	38	.019	78	--	17
	1.00	1.00		.974		.968		1.00	
	.98	--		.013		.013		--	

Table 8. Genetic variability estimates for populations of D. immigrans.

	1591				KP				SB				Korea			
Locus	N	No. of alleles	exp. h	obs. h	N	No. of alleles	exp. h	obs. h	N	No. of alleles	exp. h	obs. h	N	No. of alleles	exp. h	obs. h
ACPH	99	2	.356	.303	111	2	.386	.288	103	2	.405	.447	41	2	.115	.122
APH	56	4	.254	.267	96	3	.359	.344	24	2	.116	.125	17	3	.335	.353
ALDOX-1	37	3	.151	.162	78	4	.136	.115	92	3	.073	.076	24	2	.152	.167
ALDOX-2	--	--	--	--	79	3	.287	.278	92	3	.200	.185	39	3	.091	.179
EST-1	27	1	0	0	98	3	.018	.020	99	2	.010	.010	39	3	.190	.205
EST-2	27	1	0	0	34	2	.029	.029	30	1	0	0	40	1	0	0
EST-6	--	--	--	--	106	2	.018	.019	47	2	.041	.043	41	1	0	0
LAP-1	--	--	--	--	29	3	.067	.069	--	--	--	--	21	2	.091	.095
LAP-2	90	3	.398	.367	52	3	.501	.519	28	3	.426	.429	38	3	.341	.289
GOT-1	91	3	.019	.022	--	--	--	--	--	--	--	--	--	--	--	--
GOT-2	40	3	.093	.100	20	3	.184	.200	32	3	.090	.094	41	3	.138	.122
ODH	29	2	.066	.069	92	2	.043	.022	87	2	.033	.034	24	2	.041	.042
PGM	8	1	0	0	38	3	.050	.053	78	3	.062	.064	17	1	0	0
Ave. No. of Alleles		1.64				2.24				1.94				1.82		
No. of loci Analyzed		(14)				(17)				(16)				(17)		
$\bar{H}$			.092				.115				.100				.093	

Table 9. Comparison of enzyme variability in exotic and endemic Hawaiian Drosophilidae.

Species	Locale	No. of loci examined	Average no. of alleles/locus*	Proportion of loci polymorphic*	$\bar{H}$
D. simulans	Hawaii	15	1.60	.400	.073
D. simulans	Oahu	15	1.33	.333	.073
D. simulans	Texas[a]	18	2.17	.556	.162
D. immigrans	Hawaii	17	2.24	.706	.115
D. immigrans	Korea	17	1.82	.529	.093
D. grimshawi	Molokai[b]	14	2.14	.786	.129
D. engyochracea	Hawaii[c]	20	1.36	.300	.128
D. silvestris	Hawaii[d]	12	1.58	.750	.117
D. mimica	Hawaii[c]	21	2.29	.476	.194
D. silvarentis	Hawaii[e]	12	--	.416	.080
D. sproati	Hawaii[e]	15	1.73	.467	.083
D. orthofascia	Hawaii[e]	13	1.46	.308	.025

* at least 1% polymorphism
a Kojima et al. 1970
b Steiner et al. 1973
c Steiner 1974
d Johnson et al. 1974 (EST loci excluded)
e Steiner, unpublished observations, N > 50 in all cases

D. simulans can carry high levels of genetic variability (Kojima et al. 1970) has some bearing on this point. Both the present study and that of Kojima et al. are based on samples drawn directly from nature. The studies which are at odds with these results were drawn mostly from strains kept in the laboratory for many months prior to analysis. Lower levels of variability in such strains might result from an inbreeding effect or from genetic drift occurring during establishment of the lines. Another hypothesis might be that populations of D. simulans occurring in northerly latitudes (from which many of the strains in Berger's 1971 study are drawn) have a paucity of genetic variation. Alternatively, we can explain the difference between the studies by asking why genetic variation in southerly populations of D. simulans is higher. It is known that D. simulans occurs in higher frequencies than its sibling species D. melanogaster in populations nearer the equator while the reverse seems true for northerly populations in which these species are sympatric and therefore potential competitors. Kojima et al. (1970) note that very few D. melanogaster were observed in their study, an observation similar to ours. Relieved competitive pressure might be indicated as having some favorable effect on genetic variability in D. simulans. Population cage studies might provide an answer to the effects of competition on electrophoretic variability in these species. However, Gibo (1972) has demonstrated that D. simulans may be favored in competition with D. melanogaster under certain conditions, indicating environment may play a significant role in competing species. Relationships between competitive ability and environment must be defined to obtain an accurate picture.

Another hypothesis might be based on the continuous introduction of the species from other parts of the world. Such continual recolonization is bound to introduce genomes differing in the extent and possibly types of their allelic contents. This may be the basis for the allelic differences between Texas and Island populations of D. simulans, since the Hawaii populations are expected to experience recruitment from Asian populations as well as Nearctic and South American populations. Analysis of Asian populations would throw some light on the validity of this hypothesis. A third hypothesis is that mutation at all loci after an initial founding could have resulted in the levels of variability discerned in the island populations. This does not explain the levels of variability observed in the Austin, Texas samples which at least in theory may be continuous with northerly populations. It also would predict that we might expect

more variability than actually observed, since it is likely that D. simulans has been a colonizer since the first Polynesians arrived with their fruits and animals approximately 2 millenia ago. Their populations even in the island environments appear to be much larger than that of the endemic Hawaiian drosophilid fauna. The theory discounts, however, the fact that mutation would likewise be occurring in the northerly populations at the same rates. We would have to hypothesize a second condition concerning the effect of ecological heterogeneity on the phenotypes being produced via mutation. We would expect that more niches exist in tropical areas and Levene's hypothesis (1953) could be applied to support our theory. In fact Heed (1968) has demonstrated that D. simulans has apparently moved into at least one endemic niche on Hawaii and two on Oahu. How many others it presently occupies is unknown. Our theory breaks down at this point, however, since Levene's hypothesis is enough to account for the observed variability independently of any theory concerning mutation rates. Of critical importance here is the observation that allele homologies have not been drawn between the continental and island types, a factor having some bearing on any hypothesis that is advanced to explain the discrepancies in genetic variation between this and the other two studies (Berger 1971, O'Brien and MacIntyre 1969).

With respect to D. simulans, our study indicates that chromosomal monomorphism does not necessarily imply the existence of low electrophoretic variability. Steiner (1974) has found electrophoretic variability high in the chromosomally monomorphic Hawaiian drosophila D. engyochracea as well. This does not invalidate O'Brien and MacIntyre's (1969) suggestion that genomic limits on heterozygosity may exist, but implies that it may not be true for D. simulans. It does support the idea that variable genotypes are of some value in adaptation to new environments if common links between phenotype diversity and niche exploitation could be demonstrated in D. simulans in Hawaii. It also suggests that D. simulans may not be a prototype for an all purpose genotype unless our choice of electrophoretic markers is strongly biased (which may very well be likely) or unless the Carson (1965) hypothesis is extended to include gene loci especially related to metabolic requirements of the organism in question.

To our knowledge this is the first report on extensive genic (electrophoretic) variability in D. immigrans. As in D. simulans, variability is not lacking ($\bar{H}$ = .092 - .115), and is slightly higher than in the latter. Heed (1968) has found that D. immigrans has moved into as least 7 endemic niches, all of which are

occupied to differing extents by endemic Drosophila species. Of particular interest is the niche associated with the soapberry fruit (Sapindus). This fruit has unusually high concentrations of saponin, a known toxic compound. Found only in Kipuka Ki and Kipuka Puaulu on Hawaii, it is also used as a larval substrate by the endemic drosophila D. mimica and D. kambysellisi. Thus biochemical as well as competitive adaptation must take place here. Heed's (1968) data indicate that larval development is retarded in this substrate as compared to others. In addition, chromosome studies by Paik and Sung (1972) on D. immigrans populations show that inversion frequencies differ greatly between Kipuka Puaulu and higher elevations, indicating that coadaptive processes in genetic factors may be involved as well. The relationship between the genetic variability and the niche types this species is presently occupying in the insular situation deserves further investigation.

We have been particularly interested in this study in whether any vast differences in genetic variability might be found between colonizing and endemic species. The variability exposed in the colonizing species is obviously of an intermediate value when compared to that in endemic species (Table 9). In a previous report Steiner (1974) has suggested that key components of environmental heterogeneity may be easily related to genic diversity in endemic insular species. This implies that associations to such environmental parameters may be harder to demonstrate in cosmopolitan species which represent genotypes that may meet ecological vicissitudes with differing genetic potentials and different homeostatic mechanisms. Investigating the potential for physiological and genetic response of D. simulans and D. immigrans with respect to environmental factors, such as the ability to resist desiccation or to tolerate extreme heat, should prove informative in this regard.

We cannot conclude that these cosmopolitan species are using their variability to better invade and adapt to the Hawaiian niches. Experiments would have to be specially designed to investigate this phenomenon. We demonstrated that D. simulans and D. immigrans are as variable as endemic Drosophila. This either implies that a long time has passed since founding or that many founding events have occurred. In addition, the founding environment is not without some heterogeneity, especially at microenvironmental levels; and both D. simulans and D. immigrans must experience some competition with endemic species. However, the fact that these two cosmopolitan species occur at all in apparently high numbers

indicates their success. The effect of this success on endemic Drosophila is difficult to assay.

Endemic species which may presently occupy the same niche do not appear to suffer any consequences in nature. However, we have no evidence as to what the consequences of colonization may have been at the actual time of invasion. It is likely that genetic readjustment between colonizing and endemic species would occur immediately after the event, possibly as suggested by Gibo (1972). Extinction of endemic species, if it occurs at all, is also likely to occur during this period. Obviously, in the case of D. immigrans and D. simulans, we are speaking of a series of events that occurred sometime in the past.

It is assuming too much if we expect these colonizations to eventually give rise to new species. The genetic data do not support this thesis for present day populations which are widely separated geographically. The nature of both species is such that continual reintroduction may be expected. Such reintroduction necessarily means that the Hawaii populations of D. simulans and D. immigrans must be able not only to accommodate this new source of genetic material, but to utilize it for conservative reasons as well. If the direction of gene flow is one-way, as this concept implies, it means that any barriers that arise will not be between incoming migrants and the existing Hawaiian populations. The concept suggests that continual enrichment of an insular gene pool can result on a rare but perpetual basis. Such renewal can serve a long-term evolutionary purpose as an additional source of genetic variation over and above that of mutation. Such links to cosmopolitan populations would prevent the factors from arising which Carson (1968) hypothesizes to occur if speciation is to take place. It gives a cosmopolitan species a definite advantage over endemic life forms in its adopted ecosystem.

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